FURTHER CHARACTERIZATION OF THE INHIBITORY EFFECTS OF NUCLEOTIDES ON CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously shown that nucleotides inhibit the secretion of chloride by the isolated perfused rectal gland (Silva P, et al. Bull. MDIBL 33:75, 1994). In these experiments purinergic nucleotides (and their derivatives) were more potent than UTP. Because of the presence of ecto-5'-nucleotidases in the rectal gland purinergic nucleotides are hydrolyzed to adenosine which has its own independent inhibitory effect. The present experiments were done in order to examine further the possible role of adenosine as a mediator of the inhibitory effect of nucleotides.

Shark rectal glands were perfused as described in Silva P, et al. Methods Enzymol. Vol 192:754-66, 1990. The glands were perfused with theophylline 2.4 x 10^{-4}M throughout the experiment. To determine whether ATP in the perfusate was hydrolyzed to adenosine by the ecto-5'-nucleotidases of the gland we measured adenosine in the venous effluent. The effluent was collected before and during the perfusion with the nucleotide in graduated tubes on ice and immediately frozen. Adenosine was measured using adenosine deaminase and following the decrease in absorption at 247 nm in a Beckman DU spectophotometer. Glands were perfused with ATP at a concentration of 10^{-5}M and 10^{-4}M and with ß, γ -methylene ATP 10^{-4}M. The concentration of adenosine in the perfusate prior to the addition of ATP was less than 10^{-6}M. Perfusion with ATP 10^{-5}M produced a concentration of 0.95±0.14 x 10^{-5}M adenosine in the venous effluent (n=6). Perfusion with ATP 10^{-4}M produced adenosine concentrations of 0.48±0.06 x 10^{-4}M (n=6). Thus, ATP is completely converted to adenosine at an ATP concentration of 10^{-5}M, while at a concentration of 10^{-4}M approximately 50% of the ATP is converted. On the other hand, ß, γ -methylene ATP, 10^{-5}M, which is resistant to ecto-5'-nucleotidases, was not hydrolyzed to adenosine.

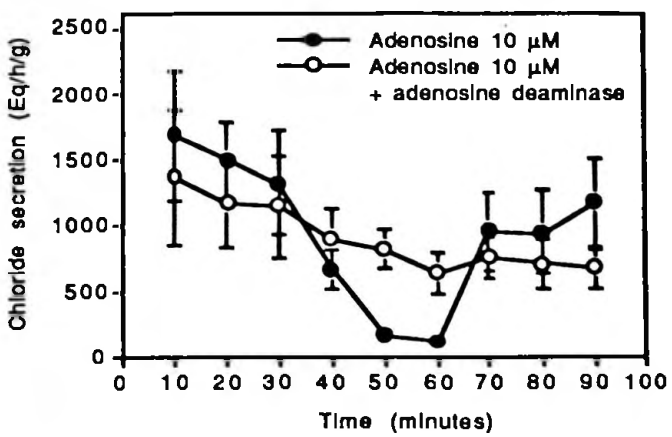

Figure 1. Isolated rectal glands perfused with theophylline throughout the whole experiment and adenosine or adenosine plus adenosine deaminase between 30 and 60 minutes. Adenosine deaminase completely prevents the inhibitory effect of adenosine.

To determine whether these concentrations of adenosine were capable of inhibiting chloride secretion in glands perfused with theophylline, we perfused glands with adenosine and theophylline 2.4 x 10^{-4}M, in the presence and absence of adenosine deaminase 0.6 U/ml. Figure 1 shows that adenosine 10^{-5}M inhibited the secretion of chloride while adenosine deaminase completely prevented this effect. A similar inhibition was observed with 10^{-5}M adenosine. In contrast (Figure 2), 10^{-5}M ß, γ -methylene ATP inhibited chloride secretion both in the presence and absence of adenosine deaminase.

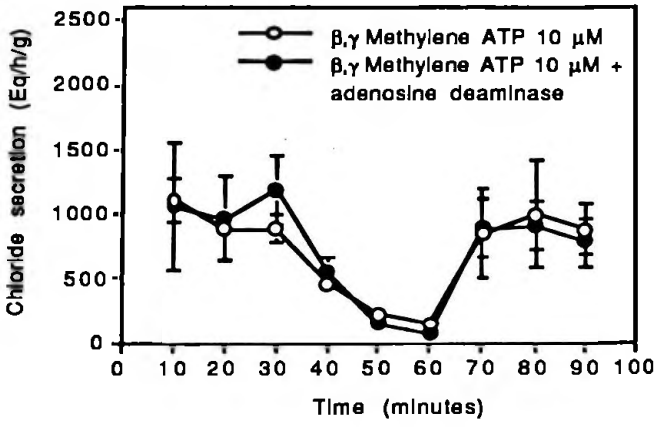

Figure 2. Isolated rectal glands perfused with theophylline throughout the whole experiment and ß, γ-methylene ATP or ß, γ-methylene ATP plus adenosine deaminase between 30 and 60 minutes. Adenosine deaminase did not prevent the effect of ß, γ-methylene ATP.

These experiments indicate that 1) extracellular ATP is rapidly converted to adenosine by the rectal gland presumably by ecto-5'-nucleotidases, 2) the inhibitory effect of adenosine on glandular secretion, thought to be mediated by a1 adenosine receptors, is not blocked completely by theophylline 2.4 x 10^{-4}M, and 3) intact nucleotides that are not converted to adenosine exert an inhibitory action on rectal gland secretion.

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